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Gsα Mutations in Hyperfunctioning Thyroid Adenomas

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Hyperfunctioning thyroid adenomas are benign tumors characterized by their autonomous growth and functional activity, which frequently cause clinical hyperthyroidism and show a predominant radioactive iodine uptake in the nodule. Activating mutations in the gene encoding the α subunit of the stimulatory G protein (Gsα), as well as activating mutations in the gene encoding thyrotropin receptor in hyperfunctioning thyroid adenomas, have been reported. The mutations in Gsα involved the replacement of either arginine 201 with cysteine or histidine, or glutamine 227 with arginine or leucine. These residues are involved in GDP/GTP binding of Gsα and these mutations inhibit intrinsic GTPase activity that results in constitutive activation of adenylyl cyclase. The pathophysiological roles of these mutations in the formation of hyperfunctioning thyroid adenoma have been suggested. © 2000 IMSS. Published by Elsevier Science Inc.

Key Words: G protein, Thyrotropin receptor, Cyclic AMP, Adenylyl cyclase.

Introduction

G proteins are heterotrimeric enzymes that couple a variety of membrane receptors to effector molecules that mediate the cell response. G proteins are linked not only to the adenylyl cyclase system but also to ion-channels, phospholipase, and other second messengers (1–3). In the thyroid tissue, thyrotropin (TSH) binds to TSH receptor, which is composed of a large extracellular domain and a seven transmembrane-spanning domain, thereby activating the adenylyl cyclase system and phospholipase C-diacylglycerol-inositol phosphate-Ca^{2+} signaling cascade via coupling to the G proteins (4).

Hyperfunctioning thyroid adenomas are benign tumors characterized by their autonomous growth and functional activity, which frequently cause clinical hyperthyroidism. Recently, activating mutations in the gene encoding the α subunit of the stimulatory G protein (Gsα), as well as activating mutations in the gene encoding TSH receptor in hyperfunctioning thyroid adenomas have been reported (5–17). After a brief overview of G protein structure and function, pathophysiology of mutations in Gsα in hyperfunctioning thyroid adenomas will be discussed.

G Protein Structure and Function

G proteins are heterotrimers consisting of a guanine nucleotide-binding α subunit and a dimeric subunit, which includes a β- and a γ-chain. Figure 1 illustrates the cycle of G protein activation and deactivation that transmits the signal from receptors to effectors. In the inactive state, the three subunits form an αβγ heterotrimer, with the α subunit simultaneously bound to a molecule of guanosine diphosphate (GDP). GDP-bound α subunit can interact with receptors, but the association is greatly enhanced by βγ. Agonist-activated receptors act catalytically to release GDP from the α subunit and permit guanosine triphosphate (GTP) to bind to the α subunit. Binding of GTP induces a conformational change of the α subunit, leading to a decreased affinity for both the receptor and the βγ dimer and an increased affinity for a specific intracellular effector. Both GTP-bound α subunit and the βγ dimer interacted with and modulated the activity of certain effectors (2,3). The activated state lasts until the GTP is hydrolyzed to GDP by the intrinsic GTPase of the α subunit. Presence of GDP inactivates the α subunit and allows the α and βγ subunits to reassociate and return to the receptor. The inactive αβγ heterotrimer awaits an ac-

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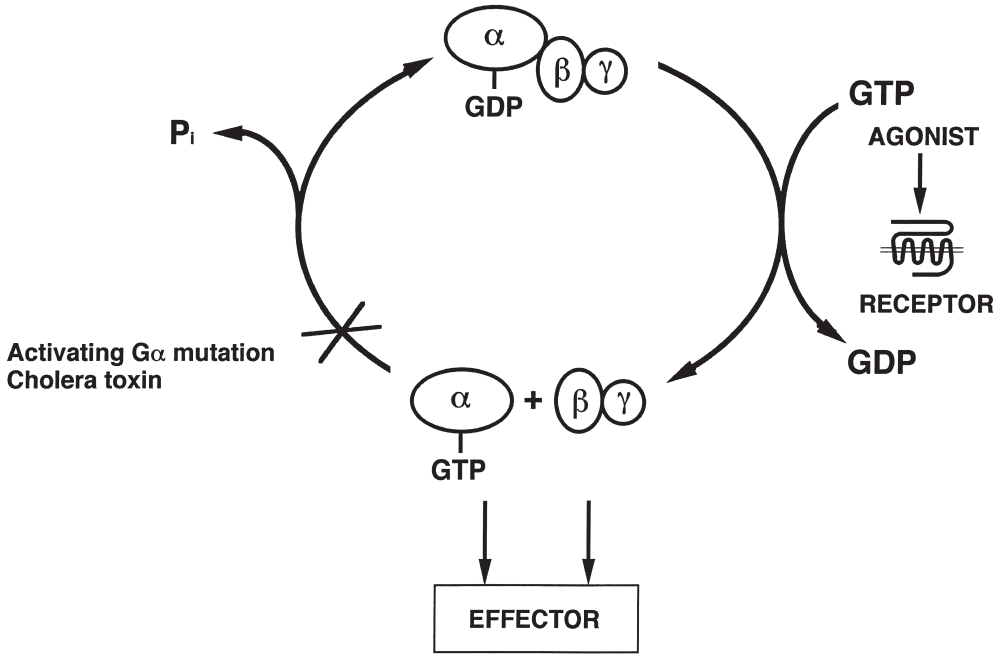

Figure 1. The G protein GTPase cycle. In the inactive state, the three subunits form an αβγ heterotrimer, with the α subunit simultaneously bound to GDP. Agonist-activated receptors release GDP from the α subunit and permit GTP to bind to the α subunit. Binding of GTP induces a conformational change of the α subunit, leading to decreased affinity for both the receptor and the βγ dimer. Both GTP-bound α subunit and βγ dimer interact with and modulate the activity of certain effectors. The activated state lasts until the GTP is hydrolyzed to GDP by the intrinsic GTPase of the α subunit. Presence of GDP inactivates the α subunit and allows the α and βγ subunits to reassociate and return to the receptor. Certain mutations in a subunit gene reduce the α subunit's intrinsic GTPase activity, locking the α subunit in the activated state and causing constitutive activation. Cholera toxin also causes constitutive activation by inhibiting GTPase activity of αs.

tivated receptor to re-enter another round of the cycle. Although the βγ subunit does not bind GTP, its active period depends on the rate of GTP hydrolysis by the α subunit. The turn-off of G protein signaling pathways *in vivo* occurs 10- to 100-fold faster than the rate of GTP hydrolysis *in vitro*, suggesting the existence of proteins that are able to increase GTP hydrolysis and return the α subunit to its inactive state. Recently, a family of GTPase-activating proteins termed RGS (for regulator of G protein signaling), which deactivates G proteins by allowing inactive heterotrimers to reform, has been identified (18,19). Certain mutations in α subunit gene reduce the α subunit's intrinsic GTPase activity, effectively locking the α subunit in the activated state and causing constitutive activation. Cholera toxin, one of the well-known bacterial toxins, also causes constitutive activation by inhibiting GTPase activity.

G proteins are defined by their α subunits. To date, at least 16 distinct α subunits have been cloned and the proteins can be divided into the following four families based upon sequence similarity: αs, αi, αq, and α12. All α subunits share at least 40% homology in amino acid sequence. These conserved regions correspond to five discrete regions (termed G boxes) that form the guanine nucleotide-binding pocket in the 3-D structure (20). The amino- and carboxy-termini, as well as the regions inserted between the G boxes, show substantial sequence divergence, which may confer specificity in G protein coupling to receptors and effectors. Regions near the carboxy-terminus have been identified as critical for both effector and receptor interactions. The amino-terminus is required for association with the βγ dimer. As discussed below, certain residues have been shown to be crucial for α subunit GTPase activity. Arginine and glutamine residues (201 and 207, respectively, in the long form of Gsα) are conserved in α subunits and are shown to be critically involved in guanine nucleotide binding.

When human thyroid membranes are stimulated with bovine TSH, the TSH receptor is able to couple to at least 10 different G proteins belonging to all four G protein families (Gs, Gi, Gq, and G12) as illustrated in Figure 2 (21). Activation of Gs results in the stimulation of adenylyl cyclase activity and the production of the second messenger cAMP. When human thyroid slices were pretreated with pertussis toxin, which leads to uncoupling of Gi family members from the receptor, the TSH receptor-mediated accumulation of cAMP further increased (21). These observations indicate that the TSH receptor is also coupled to Gi, which results in the inhibition of adenylyl cyclase. Activation of Gq results in the activation of the phospholipase C-diacylglycerol-inositol phosphate-Ca^{2+}signaling cascade. Although the effector for G12 is not well understood (3), it may be re-

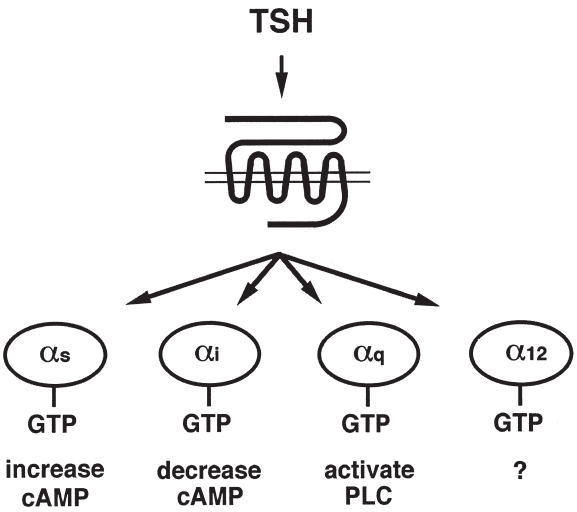

Figure 2. Multiple coupling of TSH receptor to different G protein families. TSH receptor is able to couple to at least 10 G proteins belonging to all four G protein families (αs, αi, αq, α12). PLC, phospholipase C.

lated to either growth or differentiation of thyrocytes (21). Thus, TSH receptor is suggested to be a naturally occurring receptor that can activate multiple signaling pathways (21). Although it was initially suggested that the βγ dimer is only a negative regulator in G protein-mediated signal transduction, recent evidence suggests that the βγ dimer, as well as the α subunit, positively regulates certain effectors. The βγ dimer has been shown to be a positive regulator of K^+ channel, adenylyl cyclase, phospholipase Cβ, phospholipase A2, phosphoinositide 3-kinase, and β adrenergic receptor kinase. The βγ dimer may also act through Ras to activate mitogen-activated protein (MAP) kinase pathways (2,3).

Gsα functions by coupling a number of membrane receptors with one or more isoforms of adenylyl cyclase to produce cAMP. The subsequent activation of cAMP-dependent protein kinase A (PKA) mediates most of the effects of cAMP that involve biological phenomena as diverse as metabolic and secretory pathways, differentiation, and cell growth. Cyclic AMP response element binding protein (CREB), once phosphorylated by PKA, can act in the nucleus to modulate the transcription of cAMP-responsive genes by binding to cAMP response element (CRE). The transduction of extracellular signals with mitogenic potential was thought to involve the growth factor receptor tyrosine kinase pathway and the phosphatidyl inositol-protein kinase C cascade. However, much evidence suggests the cAMP cascade positively regulates cell proliferation in certain cell types, including thyroid cells (22). The impact of cAMP pathway dysregulation on cell growth and function has been recently emphasized by evidence that naturally occurring activating mutations of both Gsα and Gs-coupled receptors are associated with tumor formation in humans.

Table 1. Prevalence of Gsα mutations in hyperfunctioning thyroid adenomas

Authors	Year	Reference	Mutations	
Lyons et al.	1990	(5)	1/4	25%
O'Sullivan et al.	1991	(6)	5/13	38%
Russo et al.	1995	(7)	9/37	24%
Du Villard et al.	1995	(8)	9/28	32%
Hamacher et al.	1995	(9)	1/4	25%
Tanaka et al.	1996	(10)	1/28	4%
Parma et al.	1997	(11)	2/33	6%
Führer et al.	1997	(25)	0/31	0%
Pinducciu et al.	1998	(26)	0/15	0%
Kamiya et al.	1998	(12)	1*	

Note: Case report.
[a]Positive tumors/studied tumors and percentage of mutations.

Gsα Mutations in Hyperfunctioning Thyroid Adenomas

The earliest evidence for the activating mutations in G protein was the discovery of constitutively elevated adenylyl cyclase and Gs activities in growth hormone-secreting pituitary tumors (23). It was then demonstrated that the same tumors contained somatic mutations in the Gsα gene (24). Following these observations, activating mutations in Gsα gene were first demonstrated in hyperfunctioning thyroid adenoma in 1990 (5). These mutations involved the replacement of either arginine 201 with cysteine (R201C) or histidine (R201H), or glutamine 227 with arginine (Q227R), or leucine (Q227L) in Gsα. As mentioned previously, these residues are involved in GDP/GTP binding, and mutation of these residues inhibits intrinsic GTPase activity, causing constitutive activation of adenylyl cyclase. Arginine 201 is also the target for the cholera toxin-catalyzed ADP ribosylation of Gsα. The results of subsequent studies have shown that activating Gsα mutations are present in 0–38% of hyperfunctioning thyroid adenomas, as summarized in Table 1 (5–12,25,26). The same mutations have also been found less frequently in other types of thyroid tumors, including nonfunctioning adenoma and cancer (27–33). In 1993, activating mutations in TSH receptor gene, which also resulted in constitutive activation of adenylyl cyclase, were demonstrated in hyperfunctioning thyroid adenomas (13). Subsequent studies have shown that activating mutations in the TSH receptor gene are present in 0–82% of hyperfunctioning thyroid adenomas (7,11,13–17,25,26,34). Thus, hyperfunctioning thyroid adenomas may be caused by either activating TSH receptor mutations or by activating Gsα mutations. Although it has been reported that the former may predominate (16), a study of 37 hyperfunctioning thyroid adenomas found three TSH receptor mutations and nine Gsα mutations (7).

The results of the analysis of hyperfunctioning thyroid adenomas have shown that the incidence of TSH receptor mutations or Gsα mutations is widely different in the literature. It might be argued that methodological differences are

responsible for the discrepancies of the results in different laboratories. Alternatively, genes encoding other proteins of TSH receptor-dependent signaling may be mutationally activated in hyperfunctioning thyroid adenomas, in which no mutations of TSH receptor or Gsα are detected. As an alternative hypothesis, the occurrence of activating mutations in the TSH receptor or Gsα gene may be related to iodine deficiency (35). Whereas substantial numbers of hyperfunctioning thyroid adenomas harbor activating TSH receptor or Gsα gene mutations in regions with iodine deficiency, these mutations are very rare in countries with high iodine uptake of the population, e.g. Japan (10,34). It is not known whether these mutations are the consequence of chronic growth stimulation of iodine-deficient thyroid gland, which thereby is hit by an increased number of mutational events, or whether intracellular iodine deficiency itself causes a mutation-favoring environment (35).

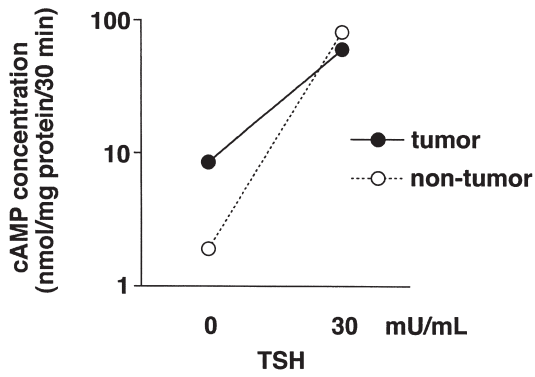

Figure 3. cAMP concentration (nmol/mg protein/30 min) in cultured cells from non-tumor or tumor region with or without 30 mU/mL TSH. Cells were incubated for 30 min in HEPES-buffered medium containing 0.1 mM RO 20-1724 with or without 30 mU/mL TSH. cAMP was extracted from cells by 0.1 N HCl and was measured by a radioimmunoassay. Modified from Reference 12.

Pathophysiology of Hyperfunctioning Thyroid Adenomas with Gsα Mutations

Primary culture of thyroid cells from hyperfunctioning thyroid adenoma with Gsα mutation. To clarify the relationship between cellular functions and genetic abnormalities in hyperfunctioning thyroid adenomas, it appears necessary to characterize tumor cells by both primary culture and genetic analysis. We had the opportunity to examine the function of cultured cells from hyperfunctioning thyroid adenoma and its surrounding thyroid tissue, and determined the nucleotide sequences of genes encoding Gsα and TSH receptor in its tumor tissue (12). A 50-year-old Japanese woman was diagnosed with a typical hyperfunctioning thyroid adenoma on the bases of clinical hyperthyroidism, elevated levels of serum-free thyroid hormones, suppressed serum TSH level, and a predominant ^{123}I uptake in the nodule. Thyroid tissue specimens were obtained from tumor region and its surrounding non-tumor region at the time of thyroid lobectomy of the patient. The tumor was encapsulated and pathologically diagnosed as follicular adenoma.

Primary culture of cells from the tumor tissue and its surrounding normal thyroid tissue revealed that cAMP production was constitutively activated in the cells from the tumor tissue, as shown in Figure 3. Basal cAMP production in tumor cells was 4.5 times higher than that in normal non-tumor cells. cAMP production in both non-tumor and tumor cells was significantly increased by TSH (30 mU/mL); cAMP production in tumor cells in the presence of TSH was 75% of that in non-tumor cells. cAMP production in tumor cells was increased only 7.15 times from its basal level by TSH, whereas the cAMP production in non-tumor cells was increased 42.5 times from its basal level by TSH. We have also studied intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) in cultured cells from both tumor and non-tumor tissue. $[Ca^{2+}]i$ was demonstrated as suppressed, both at the basal level and in the response to TSH stimulation in cells from tumor tissue compared to those from non-tumor tissue.

Nucleotide sequence analysis of exons 8 and 9 of Gsα gene in tumor tissue and surrounding normal tissue revealed the presence of one base substitution at codon 201 (CGT to CAT) in exon 8, which resulted in replacement of arginine with histidine in tumor tissue, as demonstrated in Figure 4. No mutation was observed in the transmembrane region of TSH receptor gene and in exon 9 of Gsα gene.

These results suggest that cAMP regulatory cascade is constitutively activated, while phospholipase C-Ca^{2+} signaling cascade is suppressed in hyperfunctioning thyroid adenoma with an activating mutation of Gsα gene in the present case. These observations suggest that mitogenic activity of Gsα mutation may be related to constitutive activation of the cAMP regulatory cascade but not to the phospholipase C-Ca^{2+} signaling cascade in these cells. Decreased $[Ca^{2+}]i$ in tumor cells may result from the inhibition of phospholipase C activity by increased intracellular cAMP concentration. This speculation is supported by recent evidence that elevated levels of cAMP are able to inhibit the activity of G protein-coupled phospholipase C activity in FRTL-5 rat thyroid cells transfected with cholera toxin A1 subunit (36). As previously mentioned, stimulation of human TSH receptor is known to activate the phospholipase C-diacylglycerol-inositol phosphate-Ca^{2+} signaling cascade, as well as the cAMP regulatory cascade (37,38). Transfection studies suggested that the activating mutations identified in the TSH receptor gene resulted in constitutive stimulation of the cAMP regulatory cascade and constitutive activation of the phospholipase C regulatory cascade, possibly depending on the position of mutations (16,39). Thus, one might assume that intracellular Ca^{2+} concentrations in hyperfunctioning thyroid adenomas with activating mutations in Gsα are different from those with activating mutations in TSH

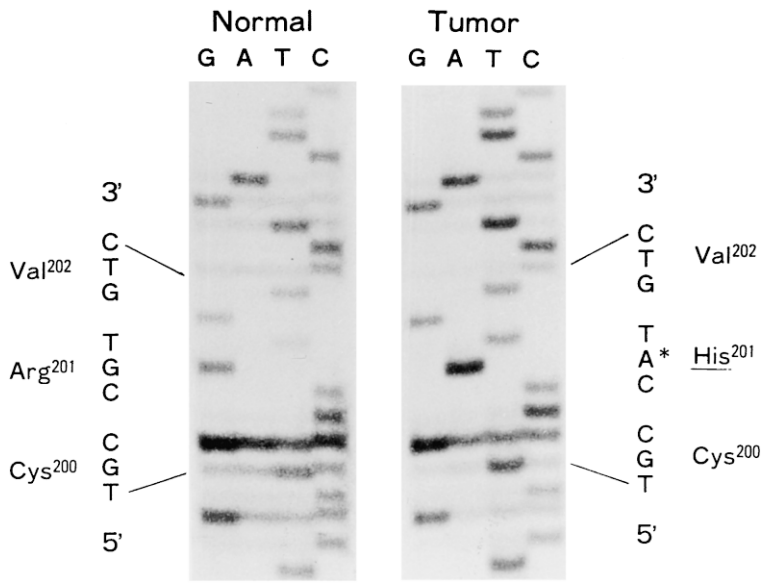

Figure 4. Nucleotide sequence analyses of exon 8 of Gsα gene in normal non-tumor tissue and tumor tissues. Nucleotide sequences of codons 200–202 are shown. Genomic DNA was extracted from normal non-tumor and tumor tissues. PCR amplified fragment of exon 8 of Gsα was subcloned and clones possessing the insert were sequenced. Reprinted from Kamiya Y, Murakami M, Yanagita Y, Koitabashi H, Nagamachi Y, Hosoi Y, Ogiwara T, Mizuma H, Iriuchijima T, Mori M. Primary culture of cells from hyperfunctioning thyroid adenoma with an activating mutation of Gαs. Mol Cell Endocrinol 1998;138:137–142, with the permission of Elsevier Science (Reference 12).

receptor, although the differences in clinical manifestations between those hyperfunctioning thyroid adenomas are not known.

Gsα expression in hyperfunctioning thyroid adenomas. An increased amount of Gsα has recently been reported in hyperfunctioning thyroid adenomas (9) and thyroid tumors with activating mutations in Gsα (40). It was suggested that increased level of cAMP concentration resulted in activation of Gsα synthesis (40), supported by previous results demonstrating cAMP stimulation of Gs protein in cultured pig thyroid cells (41). Although the specific mechanisms might regulate Gsα expression in thyroid cells, the results obtained in pituitary tumors indicate that over-expression of the mutant protein is not required for the full biochemical and oncogenic effects of Gsα mutations (23,24).

Pathophysiological Roles of Mutant Gsα in Hyperfunctioning Thyroid Adenomas

Pathophysiological roles of elevation of cAMP levels by constitutive activation of adenylyl cyclase in mitogenesis or oncogenesis of the thyroid are still controversial. As discussed subsequently, pathophysiological roles of constitutive activation of adenylyl cyclase have been studied using various experimental models, including FRTL-5 rat thyroid cells transfected with mutant Gsα or cholera toxin A1 subunit; human thyrocytes transfected with mutant Gsα; and transgenic mice that overexpress mutant Gsα, A2 adenosine receptor or cholera toxin A1 subunit in their thyroid glands.

FRTL-5 rat thyroid cells transfected with mutant Gsα or cholera toxin A1 subunit. The introduction of the Q227L Gsα mutation in FRTL-5 rat thyroid cells caused stimulation of adenylyl cyclase activity and intracellular accumulation of cAMP (42). It is noteworthy that these cells showed a TSH-independent proliferation, suggesting that activating mutation in Gsα is sufficient to proliferate FRTL-5 cells (42). A counteracting mechanism has been suggested in FRTL-5 cells expressing a constitutively active mutant Gsα. The expression of Q227L Gsα in FRTL-5 cells is accompanied by increased cAMP hydrolysis, accounted for at least in part by constitutive induction of a specific phosphodiesterase (PDE) form, PDE4D2 (43). This enzyme is expressed in normal cells only after TSH stimulation. A compensatory increase in PDE activity may be a general feedback mechanism in which cells activate in response to elevations in cAMP. However, in FRTL-5 cells, the PDE system can only attenuate the phenotype induced by activating mutations in Gsα (43). FRTL-5 cells transfected with a transgene in which the cholera toxin A1 subunit, which inhibits GTPase activity, is expressed under the control of the rat thyroglobulin gene promoter caused stimulation of baseline cAMP levels and adenylyl cyclase activity (36). Interestingly, implantation of these cells onto nude mice resulted in enhanced cell proliferation and neoplastic transformation (44). These results suggest that activation of Gsα and con-

stitutive production of cAMP in FRTL-5 cells can result in TSH-independent cellular proliferation and neoplastic transformation.

Human Thyrocytes Transfected with Mutant Gsa. Expression of mutant Gsα driven by a strong retroviral vector in human thyrocytes failed to promote cell growth, suggesting that the activating mutations in Gsα may not be a sufficient proliferogenic stimulus in itself to account for the formation of hyperfunctioning thyroid adenoma (45). These results were somewhat different from results obtained with FRTL-5 cells. Because FRTL-5 cells are an immortalized cell line already altered by other genetic events, these results support the concept that additional alterations other than Gsα mutations may be a prerequisite for nodular transformation and tumor growth. To support this concept, a recent clinical report suggests that elevation of cAMP alone may not be sufficient to induce thyroid neoplasia (46).

Transgenic mouse models. Three transgenic mouse models using thyroglobulin promoter for thyroid specific gene expression that examine the oncogenic potential of constitutively elevated cAMP levels in the thyroid have been developed as follows: one model in which Gsα with an activating mutation (R201H) is expressed (47); one in which the A2 adenosine receptor, which activates adenylyl cyclase via coupling to the Gs protein, is overexpressed (48), and one in which the cholera toxin A1 subunit is expressed (49). Transgenic mice expressing Gsα with an activating mutation develop hyperfunctioning thyroid adenomas associated with elevated cAMP levels and increased radioactive iodine uptake, as well as elevated serum triiodothyronine (T_3) and thyroxine (T_4) levels (47). Thus, it appears that mutant Gsα expression in the thyroid is oncogenic in transgenic mice. However, although expression of the transgene at the mRNA level could be detected in the thyroid glands of 4-month-old transgenic mice, histological abnormalities and elevated T_3 and T_4 levels did not occur before 8 months of age. Thus, the authors concluded that considering the age of onset and the focal nature of the lesions seen in transgenic mice, mutant Gsα alone might be insufficient to produce benign tumors or even hyperplasia (47). Transgenic mice overexpressing the A2 adenosine receptor develop thyroid hyperplasia and hyperthyroidism in association with a constitutively activated cAMP cascade (48). In those animals, no obvious signs of malignancy could be found in the thyroid, although progressive heterogeneity of the tissue in older mice and presence of dense tissue nodules were observed. Transgenic mice expressing the cholera toxin A1 subunit develop thyroid hyperplasia and hyperthyroidism in association with a constitutively activated cAMP cascade. These animals showed no evidence of thyroid neoplasia (49). These transgenic mouse models suggest that activating mutations in Gsα gene in the thyroid primarily cause hyperfunction and that secondary mechanisms may be required to lead to tumor formation. Thus, mutationally activated hyperfunctioning thyrocytes may acquire additional genetic and epigenetic alterations that promote cell proliferation and result in tumor formation.

Mechanisms of Mitogenic and Oncogenic Effects of Gsα Mutations

As mentioned above, it is clear that constitutive activation of adenylyl cyclase by mutations in Gsα causes hyperfunction in hyperfunctioning thyroid adenoma. Although it is still controversial whether secondary mechanisms other than elevations in cAMP levels are required for tumor generation, it has been suggested that by phosphorylating the CREB, PKA causes transcriptional induction of genes involved in the regulation of cell growth in certain cell types (22). However, it is not clear how the cAMP signal generated by mutationally activated Gsα interacts with other mitogenic pathways. In thyroid cells, elevations in the level of cAMP act synergistically with receptor tyrosine kinases in stimulating cell growth (22). One of the major signaling pathways controlled by receptor tyrosine kinase is the MAP kinase cascade, which leads to the phosphorylation of several mitogenic substrates (50). Autophosphorylation of receptor induced by growth factor binding stimulates MAP kinases by a mechanism involving activation of Ras via the adaptor protein Grb2 and the guanine nucleotide exchange factor Sos. Ras activates the kinase activity of Raf-1, which stimulates the MAP kinase kinase MEK. Treatment with cAMP-raising agents blocks MAP kinase activation by both receptor tyrosine kinases and G protein-coupled receptors in cells in which cAMP shows an inhibitory effect on cell growth. One of the mechanisms of the cAMP action seems to be PKA-dependent phosphorylation of Raf-1, which prevents activation of Raf-1 by Ras (51). Because activation of PKA leads to inhibition of Raf-1, it is not clear how cAMP can stimulate growth in cooperation with receptor tyrosine kinases. In Swiss 3T3 fibroblasts, in which cAMP acts as a mitogenic signal, cAMP nearly abolishes epidermal growth factor-dependent Raf-1 activation but does not inhibit stimulation of MEK and MAP kinase activities (52). In certain cells, therefore, the MAP kinase system can be activated through a pathway independent of Ras and/or Raf and is not susceptible to inhibition by cAMP. It has been reported that in these systems, cAMP even slightly stimulates MAP kinase activity. In human thyroid cells, it has been reported that TSH increases cAMP levels and MAP kinase activity simultaneously (53).

Summary

Mutations affecting the signal transduction pathway and thereby constitutively activating intracellular signal transduction cascade are likely to be one of the factors in the de-

velopment of hyperfunctioning thyroid adenomas. However, the relation between the consequences of constitutively activating TSH receptor or Gsα mutations such as elevated cAMP levels and development and growth of these tumors is not completely understood and requires further studies. No obvious mutations in TSH receptor gene or Gsα gene have been detected in several cases of hyperfunctioning thyroid adenomas, especially in areas with high iodine uptake. Further studies are required to elucidate the mechanisms involved in the widely different incidence of those mutations, especially the role of the difference in iodine uptake in the mutational events in those genes. It also appears necessary to search for still unknown molecular alterations, such as other G proteins, in the pathogenesis of hyperfunctioning thyroid adenomas in which no mutations are observed in the TSH receptor or Gsα gene.

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